

*CANDIDA ALBICANS GENE, INTEGRIN-LIKE PROTEIN,
ANTIBODIES, AND METHODS OF USE*

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This application is a divisional of pending U.S. Patent Application
Serial No. 09/264,604, filed March 8, 1999, ^{now U.S. Patent 6,346,411,} which is a divisional of U.S. Patent
Application Serial No. 08/642,846, filed May 3, 1996, ^{U.S. Patent 5,886,151,} and issued March 23, 1999 as
10 U.S. Patent No. 5,886,151, both of which are incorporated herein by reference.

Statement of Government Rights

This invention was made with government support under Grant No.
R-01 AI25827, awarded by the National Institutes of Health. The government has
15 certain rights in the present invention.

Background of the Invention

Candida albicans is the leading fungal pathogen in normal hosts and
in patients with damaged immune systems. In normal hosts, disease caused by *C.*
20 *albicans* ranges from mild, easily treated, superficial disease (e.g., thrush in
newborn infants; paronychia in workers whose hands are immersed in water) to
more severe, chronic or recurrent infections (e.g., candidal vaginitis). It is estimated
that 5% of women of child-bearing age will suffer from recurrent candidal vaginitis
(Hurley, "Trends in candidal vaginitis." *Proc. R. Soc. Med.* 70 (Suppl. 4), 1-8
25 (1970), and that virtually every woman will experience at least one episode during
her reproductive years. Vaginitis is particularly frequent in otherwise normal
females with diabetes or a history of prolonged antibiotic or oral contraceptive use.
While short-term topical therapy is effective in treating individual episodes of
vaginitis, such agents do not prevent recurrences. Thus, even in the normal host,
30 infection with *C. albicans* can occur at epithelial surfaces, and recurrences are not
prevented by presently available therapies.

In immunocompromised hosts such as cancer patients, transplant

patients, post-operative surgical patients, premature newborns, or HIV-infected people, *C. albicans* ranks as the leading fungal pathogen. In this population, disease ranges from aggressive local infections such as periodontitis, oral ulceration, or esophagitis in HIV-infected patients, to complex and potentially lethal infections of the bloodstream with subsequent dissemination to brain, eye, heart, liver, spleen, kidneys, or bone. Such grave prognoses require more toxic therapy, with attendant consequences from both the underlying infection and the treatment. Here again, the infection typically begins at an epithelial site, evades local defenses, and invades the bloodstream in the face of immunosuppression. Strategies to interrupt candidal adhesion therefore have broad applicability to the prevention of mild but recurrent disease in the normal host and to the reduction of substantial morbidity and mortality in the immunocompromised.

It is well recognized that *C. albicans* adheres to epithelial and endothelial cells in the human host, oftentimes by recognizing proteins of the extracellular matrix called ligands. These ligands include proteins such as fibronectin, vitronectin, fibrinogen, the C3 degradation fragment iC3b, or the shorter C3 degradation fragment C3d. Because recognition of all of these proteins except C3d is dependent upon the amino acid sequence ARGININE-GLYCINE-ASPARTIC ACID or R-G-D, these candidal adhesions are thought to operate like the vertebrate integrins and are called "integrin-like proteins" or "integrin analogs."

Vertebrate integrins are composed of two subunits: an α -subunit and a β -subunit. There are approximately 14 α and 8 β subunits described to date in vertebrate cells. Using monoclonal or polyclonal antibodies to vertebrate integrins, several investigators have obtained evidence for integrin-like proteins in *C. albicans*: an α M analog, an α 5/ β 1 complex, or a β 1 analog. Neither the α 5/ β 1 complex nor the β 1 analog has been isolated from *C. albicans* or from any other candidal species, and the responsible genes encoding these "integrin-like proteins" have not been identified.

Summary of the Invention

The present invention provides an isolated and purified DNA molecule encoding a *Candida albicans* protein with integrin-like motifs that hybridizes to DNA complementary to DNA having SEQ ID NO:1 under the stringency conditions of hybridization in buffer containing 5x SSC, 5x Denhardt's, 0.5% SDS, 1mg salmon sperm/25 mls of hybridization solution incubated at 65°C overnight, followed by high stringency washing with 0.2x SSC/0.1% SDS at 65°C. Preferably, the present invention provides an isolated and purified DNA molecule encoding the *Candida albicans* protein with integrin-like motifs which has the amino acid sequence having SEQ ID NO:2. Preferably, the DNA is genomic DNA which has the nucleotide sequence shown in Table 1 (SEQ ID NO:1).

The present invention also provides a vector and a cell line transformed by an extrachromosomal plasmid containing non-native DNA encoding *Candida albicans* protein with integrin-like motifs (i.e., *C. albicans* integrin-like protein), as described herein. The cell line preferably comprises *S. cerevisiae*. This cell line can be used in a method of delivering a gene product to a subject.

The present invention also provides a *Candida albicans* protein with integrin-like motifs comprising an I domain, two EF-hand divalent cation binding sites, a sequence sufficient to encode a transmembrane domain, an internal RGD tripeptide, and a carboxy-terminal sequence with a single tyrosine residue. As used herein, an "internal" RGD tripeptide means that the RGD sequence is in the *Candida* protein, not in the vertebrate proteins recognized by integrins. Preferably, the isolated and purified *C. albicans* integrin-like protein has an amino acid sequence which is SEQ ID NO:2. Also provided are isolated and purified peptides, such as those having an amino acid sequence selected from the group consisting of: YLS PTN NNN SKN VSD MDL HLQ NL (SEQ ID NO:4); DWK LED SND GDR EDN DDI SRF EK (SEQ ID NO:5); SKS ANT VRG DDD GLA SA (SEQ ID NO:6); DHL DSF DRS YNH TEQ SI (SEQ ID NO:7); and WIQ NLQ EII YRN RFR RQ (SEQ ID NO:8). The invention also provides a vaccine comprising the protein and peptides, either singly or together, described herein as well as an

isolated and purified antibodies to the *C. albicans* integrin-like protein and peptides described herein.

The invention also provides a method of inhibiting adhesion of *Candida albicans* to cells (preferably epithelial cells, and more preferably human epithelial cells). The method includes contacting the *Candida albicans* with
 5 antibodies to the *Candida albicans* protein with integrin-like motifs (α Int1p) or to fragments thereof as described herein.

Brief Description of the Drawings

10 Figure 1 is a graph of the blockade of candidal adhesion to HeLa cells by antibodies to α Int1p.

Figure 2 is a graph of the blockade of candidal adhesion to CHO cells by antibodies to α Intp1.

Detailed Description

15 Specifically, the present invention is directed to the cloning and expression of a gene (*α INT1*) for an integrin-like protein (α Int1p) of *Candida albicans*. To that end, the invention provides an isolated and purified DNA molecule encoding a *Candida albicans* protein with an integrin-like motifs or biologically active derivative thereof. More preferably, the DNA is a genomic
 20 DNA molecule that encodes the protein represented by the amino acid sequence shown in Table 2 (SEQ ID NO:2). Most preferably, the genomic DNA molecule is represented by the complete nucleotide sequence shown in Table 1 (SEQ ID NO:1). Isolated and purified peptides encoded by this DNA, and derivatives thereof, which are biologically active are also within the scope of the invention.

25 As used herein, the terms "isolated and purified" refer to *in vitro* isolation of a DNA molecule or protein from its natural cellular environment, and from association with other coding regions of the *C. albicans* genome, so that it can be sequenced, replicated, and/or expressed. Preferably, the isolated and purified DNA molecules of the invention comprise a single coding region. Thus, the present
 30 DNA molecules are those consisting essentially of a DNA segment encoding an

integrin-like protein or biologically active derivative thereof. Although the DNA molecule includes a single coding region, it can contain additional nucleotides that do not detrimentally affect the function of the DNA molecule, i.e., the expression of the integrin-like protein or biologically active derivative thereof. For example, the 5' and 3' untranslated regions may contain variable numbers of nucleotides. Preferably, additional nucleotides are outside the single coding region.

The present invention also provides an isolated and purified DNA molecule that encodes integrin-like protein (α Int1p) and that hybridizes to a DNA molecule complementary to the DNA molecule shown in Table 1 (SEQ ID NO:1) under high stringency hybridization conditions. As used herein, "high stringency hybridization conditions" refers to hybridization in buffer containing 5x SSC, 5x Denhardt's, 0.5% SDS, 1mg salmon sperm/25 mls of hybridization solution incubated at 65°C overnight, followed by high stringency washing with 0.2x SSC/0.1% SDS at 65°C.

It is envisioned that oligonucleotides are also possible. Oligonucleotide probes and primers are segments of labeled, single-stranded DNA which will hybridize, or noncovalently bind, with complementary single-stranded DNA to be identified.

If desired, the probe and primer can be labeled with any suitable label known to those skilled in the art, including radioactive and nonradioactive labels. Typical radioactive labels include ^{32}P , ^{125}I , ^{35}S , and the like. Nonradioactive labels include, for example, ligands such as biotin or digoxigenin as well as enzymes such as phosphatase or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. The probe or primer may also be labeled at both ends with different types of labels for ease of separation, as, for example, by using an isotopic label at one end and a biotin label at the other end.

As used herein, the terms "protein with integrin-like motifs" and "integrin-like protein" refer to a candidal adhesin of *C. albicans*, that is expressed at the surface of *C. albicans* and allows candida to bind to epithelial cells, for

example. This initial adhesion to epithelium leads to subsequent events in the pathogenesis of invasive candidal infection (e.g., penetration of epithelial barriers and hematogenous dissemination). The unmodified protein (i.e., prior to any post-translational modification) is preferably of about 180-190 kDa, and more preferably of about 188 kDa. It includes several motifs common to α M and α X leukocyte integrins. These motifs include: (1) an Inserted domain ("I" domain) containing a conformationally dependent cation binding site (or MIDAS motif, as disclosed in Michishita et al., *Cell*, 72, 857-867 (1993)); (2) two linear divalent cation binding sites conforming to the EF-hand motif; (3) a sequence sufficient to encode a transmembrane domain; (4) a carboxy-terminal sequence with a single tyrosine residue; and (5) an internal RGD tripeptide (arginine-glycine-aspartic acid). The RGD site is at amino acids 1149-1151 in SEQ ID NO:2.

A "biologically active derivative thereof" is an integrin-like protein that is modified by amino acid deletion, addition, substitution, or truncation, or that has been chemically derivatized, but that nonetheless functions in the same manner as the protein of SEQ ID NO:2. For example, it is known in the art that substitutions of aliphatic amino acids such as alanine, valine and isoleucine with other aliphatic amino acids can often be made without altering the structure or function of a protein. Similarly, substitution of aspartic acid for glutamic acid, in regions other than the active site of an enzyme, are likely to have no appreciable affect on protein structure or function. The term "biologically active derivative" is intended to include *C. albicans* proteins with integrin-like motifs as thus modified. The term also includes fragments, variants, analogs or chemical derivatives thereof. The term "fragment" is meant to refer to any polypeptide subset. Fragments can be prepared by subjecting *C. albicans* proteins with integrin-like motifs to the action of any one of a number of commonly available proteases, such as trypsin, chymotrypsin or pepsin, or to chemical cleavage agents, such as cyanogen bromide. The term "variant" is meant to refer to a molecule substantially similar in structure and function to either the entire *C. albicans* integrin-like protein or to a fragment thereof. A protein or peptide is said to be "substantially similar" if

both molecules have substantially similar amino acid sequences, preferably greater than about 80% sequence identity, or if the three-dimensional backbone structures of the molecules are superimposable, regardless of the level of identity between the amino acid sequences. Thus, provided that two molecules possess similar activity, they are considered variants as that term is used herein even if the structure of one of the molecules is not found in the other, or if the sequences of amino acid residues are not identical. The term "analog" is meant to refer to a protein that differs structurally from the wild type *C. albicans* integrin-like protein, but possesses similar activity.

Several fragments of the protein have been prepared and can be used in vaccines or as antigens to prepare anti-peptide antibodies, which can be monoclonal or polyclonal (preferably polyclonal). A 236 amino acid sequence near the amino terminus of the gene product (α Int1p) is shown in Table 3 (SEQ ID NO:3). A 23-mer peptide encompassing the first cation-binding site is YLS PTN NNN SKN VSD MDL HLQ NL (SEQ ID NO:4). A 23-mer peptide encompassing the second divalent cation-binding site is DWK LED SND GDR EDN DDI SRF EK (SEQ ID NO:5). A 17-mer peptide spanning the RGD site and flanking residues is SKS ANT VRG DDD GLA SA (SEQ ID NO:6). A 17-mer peptide from the MIDAS motif of α Int1p is DHL DSF DRS YNH TEQ SI (SEQ ID NO:7). A 17-mer peptide from the C-terminus of α Int1p is WIQ NLQ EII YRN RFR RQ (SEQ ID NO:8).

The antibodies produced to these peptides bind to *C. albicans* blastospores, germ tubes, and hyphae, and thereby block epithelial adhesion of *C. albicans* (i.e., candida). Preferably, the antibodies are able to block *C. albicans* adhesion by at least about 30%, and preferably by at least about 50%. It is believed that this blocking activity of the initial adhesion to epithelium will reduce and even prevent subsequent events in the pathogenesis of invasive candidal infection.

The present invention also provides a vector comprising an isolated and purified DNA molecule encoding *C. albicans* protein with integrin-like motifs or a biologically active derivative thereof, preferably *C. albicans* protein with

integrin-like motifs having the amino acid sequence of SEQ ID NO:2. Preferably, the vector includes a sequence encoding the *C. albicans* protein with integrin-like motifs as well as a second DNA segment operably linked to the coding sequence and capable of directing expression of the coding region, such as a promoter region

5 operably linked to the 5' end of the coding DNA sequence. The vector can also include a DNA segment that is a selectable marker gene or a reporter gene as well as upstream untranslated sequence from the *C. albicans* gene.

The present invention also provides a cell line, preferably a *Saccharomyces cerevisiae* yeast strain transformed with an extrachromosomal

10 plasmid containing non-native DNA encoding the *C. albicans* protein with integrin-like motifs. *S. cerevisiae*, also known as brewer's yeast or baker's yeast, typically exhibits a spheroid, yeast-like form and, under certain conditions, can also exhibit a filamentous, mold-like form. The filamentous cells, which are often referred to as pseudohyphal cells, have an elongated morphology. *S. cerevisiae* (preferably

15 haploid *S. cerevisiae*), which is seldom a pathogen, transformed with the open reading frame of *αINT1*, displays germ tube-like projections referred to herein as "noses." Thus, synthesis of the *Candida* gene product α Int1p in *S. cerevisiae* induces germ tubes. Furthermore, α Int1p is surface expressed in *S. cerevisiae* and can be recognized by polyclonal antibodies to α Int1p peptides and by

20 monoclonal antibodies to vertebrate integrins. In this way, a generally harmless yeast becomes "sticky" and "nosey."

The *S. cerevisiae* yeast cells transformed by the gene described herein will adhere to epithelial surfaces as a result of expression of the integrin-like gene described herein; however, they will not invade the cells. Thus, "sticky" *S. cerevisiae* may colonize in patients at risk for *Candida* infection and thereby block

25 the adhesion sites, and reduce or eliminate the opportunity for *Candida* to adhere, colonize, and invade. Also, the "sticky" *S. cerevisiae* may function as a gene or gene product delivery system. For example, it is envisioned that a phosphate-binding protein could be delivered to the gastrointestinal tract of a patient with

30 chronic renal failure using *Saccharomyces* transformed by the integrin-like gene and

a second plasmid for expression of the phosphate-binding protein. Alternatively, a second plasmid could be used to provide a source of vaccine antigen for gastrointestinal pathogens like cholera. In the genitourinary tract, expression of spermicides by *S. cerevisiae* transformed with the *C. albicans* integrin-like gene on an extrachromosomal plasmid could provide a cheap and infrequent method of contraception. Also, synthesis of protein-based antiretroviral agents could help to reduce transmission of HIV in the birth canal.

1. Isolation of DNA

Several different methods are available for isolating genomic DNA. Most approaches begin with the purification of protein. Purified protein is then subjected to amino acid microsequencing, either directly or after limited cleavage. The partial amino acid sequence that is obtained can be used to design degenerate oligonucleotide probes or primers for use in the generation of unique, nondegenerate nucleotide sequences by polymerase chain reaction (PCR), sequences that can in turn be used as probes for screening genomic DNA libraries. Antibodies raised against purified protein may also be used to isolate DNA clones from expression libraries.

Alternatively, the sequences of DNAs for related proteins (e.g., human integrins) may be used as starting points in a cloning strategy, so-called "cloning by homology". Another way of utilizing sequence information from different species is to take advantage of shorter areas of high sequence homology among related DNAs from different species and to perform PCR to obtain "species-specific" nondegenerate nucleotide sequences. Such a sequence can then be used for library screening or even for direct PCR-based cloning. Detection of the desired DNA can also involve the use of PCR using novel primers.

Alternatively, the region encoding α Int1p may be obtained from a genomic DNA library or by *in vitro* polynucleotide synthesis from the complete nucleotide acid sequence.

Libraries are screened with appropriate probes designed to identify the

genomic DNA of interest. Preferably, for genomic libraries, suitable probes include oligonucleotides that consist of known or suspected portions of the α Int1p genomic DNA from the same or different species; and/or complementary or homologous genomic DNAs or fragments thereof that consist of the same or a similar DNA. For expression libraries (which express the protein), suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind to the α Int1p protein. Appropriate probes for screening genomic DNA libraries include, but are not limited to, oligonucleotides, genomic DNAs, or fragments thereof that consist of the same or a similar gene, and/or homologous genomic DNAs or fragments thereof.

10 Screening the genomic DNA library with the selected probe may be accomplished using standard procedures.

Screening genomic DNA libraries using synthetic oligonucleotides as probes is a preferred method of practicing this invention. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous to minimize false positives. The actual nucleotide sequence(s) of the probe(s) is usually designed based on regions of the α Int1p genomic DNA that have the least codon redundancy. The oligonucleotides may be degenerate at one or more positions, i.e., two or more different nucleotides may be incorporated into an oligonucleotide at a given position, resulting in multiple synthetic oligonucleotides.

15 The use of degenerate oligonucleotides is of particular importance where a library is screened from a species in which preferential codon usage is not known.

The oligonucleotide can be labeled such that it can be detected upon hybridization to DNA in the library being screened. A preferred method of labeling is to use ATP and polynucleotide kinase to radiolabel the 5' end of the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

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Of particular interest is the *α INT1* nucleotide sequence that encodes a full-length mRNA transcript, including the complete coding region for the gene product, α Int1p. Nucleic acid containing the complete coding region can be obtained by screening selected genomic DNA libraries using an oligonucleotide

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encoding the deduced amino acid sequence.

An alternative means to isolate the DNA encoding α Int1p is to use PCR methodology. This method requires the use of oligonucleotide primer probes that will hybridize to the DNA encoding α Int1p. Strategies for selection of PCR
5 primer oligonucleotides are described below.

2. Insertion of DNA into Vector

The nucleic acid containing the α INT1 coding region is preferably inserted into a replicable vector for further cloning (amplification of the DNA) or
10 for expression of the gene product. Many vectors are available, and selection of the appropriate vector will depend on: 1) whether it is to be used for DNA amplification or for DNA expression; 2) the size of the nucleic acid to be inserted into the vector; and 3) the host cell to be transformed with the vector. Most expression vectors are “shuttle” vectors, i.e., they are capable of replication in at
15 least one class of organism but can be transfected into another organism for expression. For example, a vector replicates in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome. Each replicable vector contains various structural components depending on its function
20 (amplification of DNA or expression of DNA) and the host cell with which it is compatible. These components are described in detail below.

Construction of suitable vectors employs standard ligation techniques known in the art. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required. Typically, the
25 ligation mixtures are used to transform *E. coli* K12 or *E. coli* XL1 Blue MRF strains 294 (ATCC 31,446) and successful transformants are selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by methods known in the art.

30 Replicable cloning and expression vector components generally

include, but are not limited to, one or more of the following: an origin of replication, one or more marker genes, an enhancer element, a promoter and a transcription termination sequence.

Vector component: origin of replication. Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast and viruses.

10 *Vector component: marker gene.* Expression and cloning vectors may contain a marker gene, also termed a selection gene or selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical
15 selection genes encode proteins that: (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, streptomycin or tetracycline; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacillus*. One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those
20 cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen.

A suitable marker gene for use in yeast is *URA3* or the *TRP1* gene present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature*, 282, 39 (1979); Kingsman et al., *Gene*, 7, 141 (1979); or Tschemper et al., *Gene*, 10, 157 (1980)).

25 The *TRP1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (Jones, *Genetics*, 85, 23 (1977)).

Vector component: promoter. Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the gene. Promoters are untranslated sequences located upstream (5') to the start

codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature. In contrast, constitutive promoters produce a constant level of transcription of the cloned DNA segment.

At this time, a large number of promoters recognized by a variety of potential host cells are well known in the art. Promoters are removed from their source DNA using a restriction enzyme digestion and inserted into the cloning vector using standard molecular biology techniques. Native or heterologous promoters can be used to direct amplification and/or expression of DNA. Heterologous promoters are preferred, as they generally permit greater transcription and higher yields of expressed protein as compared to the native promoter. Well-known promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems, alkaline phosphatase, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. Such promoters can be ligated to the DNA to be expressed using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems may contain a Shine-Dalgarno sequence for RNA polymerase binding.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bp upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is the CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be a signal for addition of the poly A tail to the 3' end of the coding sequence. All these sequences are suitably inserted into eukaryotic expression vectors. Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other

glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase and glucokinase. Other yeast promoters, which are
 5 inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization.

10 *Vector component: enhancer element.* Transcription of DNA by higher eukaryotes can be increased by inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of DNA, usually having about 10 to 300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation- and position-independent, having been found 5' and 3' to the transcription unit,
 15 within an intron as well as within the coding sequence itself. Typically, an enhancer from a eukaryotic cell virus will be used. Examples include the SV40 enhancer on the late side of the replication origin, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the
 20 DNA, but is preferably located at a site 5' of the promoter.

Vector component: transcription termination. Expression vectors used in eukaryotic host cells (e.g., yeast, fungi, etc.) can also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally, 3' untranslated regions of
 25 eukaryotic or viral DNAs. These regions can contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA.

The genetically engineered plasmid of the invention can be used to transform a host cell. As discussed above, a particularly desirable host is a eukaryotic microbe such as filamentous fungi or yeast. *Saccharomyces cerevisiae*,
 30 or common baker's yeast, is the most commonly used among lower eukaryotic host

microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*, *Kluyveromyces* hosts such as, e.g., *K. lactis*, *K. fragilis*, *K. bulgaricus*, *K. thermotolerans*, and *K. marxianus*, *yarrowia*, *Pichia pastoris*, *Trichoderma reesia*,
 5 *Neurospora crassa*, and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans*.

4. Transfection and transformation

Host cells are transfected and preferably transformed with the above-
 10 described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequence are in fact expressed. Numerous methods
 15 of transfection are known to the ordinarily skilled artisan, for example, the calcium phosphate precipitation method and electroporation are commonly used. Successful transfection is generally recognized when any indication of the operation of the vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the
 20 DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. Transformations into yeast are typically carried out according to the method of Van Solingen et al., *J. Bact.*, 130, 946 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. (USA)*, 76(8) 3829-3833 (1979). However,
 25 other methods for introducing DNA into cells such as by nuclear injection, electroporation, or protoplast fusion may also be used.

5. Cell Culture

Cells used to produce the *α INT1* gene product are cultured in suitable
 30 media, as described generally in Sambrook et al. Commercially available media

such as Hams F10 (Sigma), Minimal Essential Medium (MEM, Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM, Sigma) are suitable for culturing the host cells. These media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin' drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose, galactose, or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan. Induction of cells, to cause expression of the protein, is accomplished using the procedures required by the particular expression system selected.

The invention has been described with reference to various specific and preferred embodiments and will be further described by reference to the following detailed examples. It is understood, however, that there are many extensions, variations and modifications on the basic theme of the present invention beyond that shown in the examples and detailed description, which are within the spirit and scope of the present invention.

Examples

Isolation of the Gene *aINT1* from *Candida albicans*

DNA from spheroplasts of *C. albicans* 10261 (American Type Culture Collection) was isolated according to standard procedures as disclosed in Davis et al., *Methods Enzymol.*, 65, 404-411 (1980), digested with the restriction enzyme *Sau3AI*, and packaged in λ EMBL3 (Stratagene). Preliminary studies confirmed that a 3.5 kbp *EcoRI* fragment of *C. albicans* DNA hybridized with a 314 bp

EcoRI/SmaI DNA fragment derived from the transmembrane domain of human α M as disclosed in Hickstein et al., *Proc. Natl. Acad. Sci. USA*, 86, 257-261, 1989.

Primers for amplification of the *EcoRI/SmaI* α M DNA fragment were as follows:

upstream primer: 5' GAATTCAATGCTACCCTCAA (SEQ ID NO:9); and

5 downstream primer: 5' CCCGGGGGACCCCCTTCACT (SEQ ID NO:10).

A library enriched for 3.0-3.8 kbp *EcoRI* fragments from *C. albicans* was constructed by digestion of genomic DNA with *EcoRI* and ligation to pBluescript II SK(+). Plasmid minipreparations from a total of 200 colonies were screened by the sib selection technique for hybridization at 50°C with [³²P]-labeled

10 PCR product. Five clones were isolated from three successive screenings. Two of the five clones gave reproducible signals after hybridization with a degenerate oligonucleotide encoding a conserved sequence [KVGFFK] in the cytoplasmic domain of α X: 5' AA(AG) GT(CT) GG(AT) TT(CT) TT(CT) AA(AG) 3' (SEQ ID NO:11). Both clones contained a 3.5 kbp *EcoRI* insert and failed to hybridize

15 with a degenerate oligonucleotide from the *S. cerevisiae* gene *USO1* : 5' GAA AT(ACT) GA(CT) GA(CT) TT(AG) ATG 3' (SEQ ID NO:12).

A 500 bp *HindIII* subfragment from one of these clones was used to screen 20,000 clones from a library of *C. albicans* 10261 genomic DNA (prepared commercially from *C. albicans* DNA by Stratagene) by the plaque hybridization

20 technique as disclosed in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Lab. Press, Plainview, NY, 2nd Ed., pp.2.108-2.125 (1989). The largest hybridizing insert, a 10.5 kbp *SalI* fragment, was isolated by agarose gel electrophoresis, cloned, and sequenced.

25 *Sequence Analysis*

Both strands of the 10.5 kbp *SalI* fragment were sequenced by the method of gene walking on an Applied Biosystems Model 373 Automated Sequencer in the University of Minnesota Microchemical Facility. Nucleotide and protein sequence analyses were performed with the Genetics Computer Group (U.

30 of WI, Madison) Sequence Analysis Software Package, version 7.0. The nucleotide

sequence of the coding strand plus approximately 100 upstream nucleotides and 100 nucleotides of 3' untranslated sequence and the derived amino acid sequence (GenBank Acc. No. U35070) are shown in Tables 1 (SEQ ID NO:1) and 2 (SEQ ID NO:2), respectively. By Southern blot analysis under conditions of high stringency (hybridization at 65°C, final wash in 0.2x SSC/0.1% SDS at 65°C), this gene is present only in *C. albicans* and not in strains of *C. tropicalis*, *C. krusei*, *C. glabrata*, or *S. cerevisiae*.

TABLE 1

10	1	cccaaaaaag	ataaaataaa	aacaaaacaa	aacaaaagta	ctaacaaatt	attgaaactt
	61	ttaattttta	ataaagaatc	agtagatcta	ttgttaaaag	aatgaactc	aactccaagt
	121	aaattattac	cgatagataa	acattctcat	ttacaattac	agcctcaatc	gtcctcggca
	181	tcaatattta	attccccaac	aaaaccattg	aatttcccca	gaacaaattc	caagccgagt
15	241	ttagatccaa	attcaagctc	tgatacctac	actagcgaac	aagatcaaga	gaaagggaaa
	301	gaagagaaaa	aggacacagc	ctttcaaaca	tcttttgata	gaaattttga	tcttgataat
	361	tcaatcgata	tacaacaaac	aattcaacat	cagcaacaac	agccacaaca	acaacaacaa
	421	ctctcacaaa	ccgacaataa	tttaattgat	gaattttctt	ttcaaacacc	gatgacttcg
	481	acttttagacc	taaccaagca	aaatccaact	gtggacaaag	tgaatgaaaa	tcatgcacca
20	541	acttatataa	atacctcccc	caacaaatca	ataatgaaaa	aggcaactcc	taaagcgtca
	601	cctaaaaaag	ttgcatttac	tgtaactaat	cccgaatttc	atcattatcc	agataataga
	661	gtcgaggaag	aagatcaaag	tcaacaaaaa	gaagattcag	ttgagccacc	cttaatacaa
	721	catcaatgga	aagatccttc	tcaattcaat	tattctgatg	aagatacaaa	tgcttcagtt
	781	ccaccaacac	caccacttca	tacgacgaaa	cctacttttg	cgcaattatt	gaacaaaaac
25	841	aacgaagtca	atctggaacc	agaggcattg	acagatatga	aattaaagcg	cgaaaatttc
	901	agcaatttat	cattagatga	aaaagtcaat	ttatatctta	gtcccactaa	taataacaat
	961	agtaagaatg	tgtcagatat	ggatctgcat	ttacaaaact	tgcaagacgc	ttcgaaaaac
	1021	aaaactaatg	aaaatattca	caatttgtca	tttgctttaa	aagcaccaaa	gaatgatatt
	1081	gaaaacccat	taaactcatt	gactaacgca	gatattctgt	taagatcatc	tggatcatca
30	1141	caatcgatcat	tacaatcttt	gaggaatgac	aatcgtgtct	tggaatcagt	gcctgggtca
	1201	cctaagaagg	ttaatcctgg	attgtctttg	aatgacggca	taaaggggtt	ctctgatgag
	1261	gttgttgaat	cattacttcc	tcgtgactta	tctcgagaca	aattagagac	tacaaaagaa
	1321	catgatgcac	cagaacacaa	caatgagaat	tttattgatg	ctaaatcgac	taataccaat
	1381	aaggggacaac	tcttagtata	atctgatgat	catttggaact	cttttgatag	atcctataac
35	1441	cacactgaac	aatcaatttt	gaatcttttg	aatagtgcac	cacaatctca	aatttcgtta

TABLE 1 CONTD.

	1501	aatgcattgg	aaaaacaaag	gcaaacacag	gaacaagaac	aaacacaagc	ggcagagcct
	1561	gaagaagaaa	cttcgtttag	tgataatatc	aaagttaaac	aagagccaaa	gagcaatttg
	1621	gagtttgtca	aggttaccat	caagaaagaa	ccagttcttg	ccacggaaat	aaaagctcca
5	1681	aaaagagaat	tttcaagtcg	aatattaaga	ataaaaaatg	aagatgaaat	tgccgaacca
	1741	gctgatattc	atcctaaaaa	agaaaaatgaa	gcaaacagtc	atgtcgaaga	tactgatgca
	1801	ttgttgaaga	aagcacttaa	tgatgatgag	gaatctgaca	cgacccaaaa	ctcaacgaaa
	1861	atgtcaattc	gttttcatat	tgatagtgat	tggaaattgg	aagacagtaa	tgatggcgat
	1921	agagaagata	atgatgatat	ttctcgtttt	gagaaatcag	atattttgaa	cgacgtatca
10	1981	cagacttctg	atattatttg	tgacaaatat	ggaaactcat	caagtgaaat	aaccacaaaa
	2041	acattagcac	ccccagatc	ggacaacaat	gacaaggaga	attctaaatc	tttggaaagat
	2101	ccagctaata	atgaatcatt	gcaacaacaa	ttggagggtac	cgcatacaaa	agaagatgat
	2161	agcatttttag	ccaactcgtc	caatattgct	ccacctgaag	aattgacttt	gcccgtagtg
	2221	gaagcaaagt	attattcatc	ttttaatgac	gtgacaaaaa	cttttgatgc	atactcaagc
15	2281	tttgaagagt	cattatctag	agagcacgaa	actgattcaa	aaccaattaa	tttcatatca
	2341	atttggcata	aacaagaaaa	gcagaagaaa	catcaaattc	ataaagttcc	aactaaacag
	2401	atcattgcta	gttatcaaca	atacaaaaac	gaacaagaat	ctcgtgttac	tagtgataaa
	2461	gtgaaaatcc	caaatgccat	acaattcaag	aaattcaaag	aggtaaattg	catgtcaaga
	2521	agagttgtta	gtccagacat	ggatgatttg	aatgtatctc	aatttttacc	agaattatct
20	2581	gaagactctg	gattttaaaga	tttgaatttt	gccaactact	ccaataacac	caacagacca
	2641	agaagtttta	ctccattgag	cactaaaaat	gtcttgtcga	atattgataa	cgatcctaatt
	2701	gttgttgaac	ctcctgaacc	gaaatcatat	gctgaaatta	gaaatgctag	acggttatca
	2761	gctaataagg	cagcgccaaa	tcaggcacca	ccattgccac	cacaacgaca	accatcttca
	2821	actcgttcca	attcaaataa	acgagtgtcc	agatttagag	tgcccacatt	tgaaattaga
25	2881	agaacttctt	cagcatttagc	accttgtgac	atgtataatg	atatttttga	tgatttcggt
	2941	gcgggttcta	aaccaactat	aaaggcagaa	ggaatgaaaa	cattgccaaag	tatggataaa
	3001	gatgatgtca	agaggatttt	gaatgcaaag	aaagggtgtga	ctcaagatga	atatataaat
	3061	gccaaacttg	ttgatcaaaa	acctaaaaag	aattcaattg	tcaccgatcc	cgaagaccga
	3121	tatgaagaat	tacaacaaac	tgctctata	cacaatgcca	ccattgattc	aagtatttat
30	3181	ggccgaccag	actccatttc	taccgacatg	ttgccttatc	ttagtgatga	attgaaaaaa
	3241	ccacctacgg	ctttattatc	tgctgatcgt	ttgtttatgg	aacaagaagt	acatccgtta
	3301	agatcaaact	ctgttttggt	tcacccaggg	gcaggagcag	caactaatc	ttcaatgtta
	3361	ccagagccag	attttgaatt	aatcaattca	cctgctagaa	atgtgctgaa	caacagtgat
	3421	aatgtcgcca	tcagtggtaa	tgctagtact	attagtttta	accaattgga	tatgaatttt
35	3481	gatgaccaag	ctacaattgg	tcaaaaaatc	caagagcaac	ctgcttcaaa	atccgccaat
	3541	actgttcgtg	gtgatgatga	tggattggcc	agtgcacctg	aaacaccaag	aactcctacc
	3601	aaaaggaggt	ccatatcaag	caagcctgcc	aagctttctt	ctgcctcccc	tagaaaaatca

TABLE 1 CONTD.

3661	ccaattaaga ttggttcacc agttcgagtt attaagaaaa atggatcaat tgctggcatt
3721	gaaccaatcc caaaagccac tcacaaaccg aagaaatcat tccaaggaaa cgagatttca
3781	aaccataaag tacgagatgg tggaatttca ccaagctccg gatcagagca tcaacagcat
5 3841	aatcctagta tggtttctgt tccttcacag tatactgatg ctacttcaac gggtccagat
3901	gaaaacaaag atgttcaaca caagcctcgt gaaaagcaaa agcaaaagca tcaccatcgc
3961	catcatcatc atcatcataa acaaaaaact gatattccgg gtgttggtga tgatgaaatt
4021	cctgatgtag gattacaaga acgaggcaaa ttattcttta gagttttagg aattaagaat
4081	atcaatttac ccgatattaa tactcacaaa ggaagattca ctttaacggt ggataatgga
10 4141	gtgcattgtg ttactacacc agaatacaac atggacgacc ataatgttgc cataggtaaa
4201	gaatttgagt tgacagttgc tgattcatta gagtttattt taactttgaa ggcattcatat
4261	gaaaaacctc gtggtacatt agtagaagt actgaaaaga aagttgtcaa atcaagaaat
4321	agattgagtc gattatttgg atcgaaagat attatcacca cgacaaagtt tgtgcccact
4381	gaagtcaaa atacctgggc taataagttt gtcctgatg gttcatttgc tagatgttac
15 4441	attgatttac aacaatttga agaccaaact accggtaaag catcacagtt tgatctcaat
4501	tgttttaatg aatgggaaac tatgagtaat ggcaatcaac caatgaaaag aggcaaacct
4561	tataagattg ctcaattgga agttaaaatg ttgtatgttc cacgatcaga tccaagagaa
4621	atattacca ccagcattag atccgcatat gaaagcatca atgaattaa caatgaacag
4681	aataattact ttgaaggtta ttacatcaa gaaggagtg attgtccaat ttttaagaaa
20 4741	cgttttttca aattaatggg cacttcttta ttggctcata gtgaaatc tcataaaact
4801	agagccaaaa ttaatttata aaaagttggt gatttgattt atgttgataa agaaaacatt
4861	gatcgttcca atcatcgaaa ttccagtgat gtgttattgt tggatcatgc attcaaaatc
4921	aaatttgcta atggtgagtt gattgatttt tgtgctccta ataaacatga aatgaaaata
4981	tggattcaaa atttacaaga aattatctat agaaatcggg tcagacgtca accatgggta
25 5041	aatttgatgc ttcaacaaca acaacaaca caacaacaac aaagctccca acagtaattg
5101	aaaggtctac ttttgatttt ttttaatttta attggcaa atatgcccat tttgtattat
5161	cttttagtct aatagcggtt tctttttttc cagt

TABLE 2

30	1 MNSTPSKLLPIDKHSHLQLQPQSSSASIFNSPTKPLNFPRTNSKPSLDPN
	51 SSSDTYTSEQDQEKGEKKDTAFQTSFDRNFDLNSIDIQQTIQHQQQQ
	101 PQQQQQLSQTDNNLIDFSFQTPMTSTLDLTKQNPTVDKVNENHAPTYIN
	151 TSPNKSIMKKATPKASPKKVAFTVTNPEIHHYPDNRVEEEDQSQQKEDSV
	201 EPPLIQHWKDPQSQFNYSDEDTNASVPPTPLHTTKPTFAQLLNKNNEVN
35	251 SEPEALTDMKLKRENFSNLSLDEKVNLYLSPTNNNNSKNVSDMDSLQNL
	301 QDASKNKTNENIHNLSFALKAPKNDIENPLNSLTNADISLRSSGSSQSSL
	351 QSLRNDNRVLESVPKPKVNPGLSLNDGIKGFSDDEVESLLPRDLSRDK

TABLE 2 CONTD.

	401	LETTKEHDAPEHNNENFIDAKSTNTNKGQLLVSSDDHLDSFDRSYNHTEQ
	451	SILNLLNSASQSQISLNALEKQRQTQEQEQTQAAEPPEETSFSFSDNIKVKQ
	501	EPKSNLEFVKVTIKKEPVSATEIKAPKREFSSRILRIKNEDEIAEPADIH
5	551	PKKENEANSHVEDTDALLKKALNDDEESDTTQNSTKMSIRFHIDSDWKLE
	601	DSNDGDREDNDDISRFEKSDILNDVSQTSDIIGDKYGNSSSEITTKTLAP
	651	PRSDNNDKENSLSLEDPANNEQLQQLEVPHTKEDDSILANSSNIAPPEE
	701	LTLPVVEANDYSSFNDVTKTFDAYSSFEESSLSREHETDSKPINFISIWKH
	751	QEKQKKHQIHKVPTKQIIASYQQYKNEQESRVTSKVKIPNAIQFKKFKE
10	801	VNVMSRRVSPDMDDLNVSQFLPELSEDSGFKDLNFANYSNNTNRPRSFT
	851	PLSTKNVLSNIDNDPNVVEPPEPKSYAEIRNARRLSANKAAPNQAPPLPP
	901	QRQPSSTRSNSNKRVSFRVPTFEIRRTSSALAPCDMYNDIFDDFGAGSK
	951	PTIKAEGMKTLPMSDKDDVKRILNAKKGVTQDEYINAKLVDQKPKKNSIV
	1001	TDPEDRYEELQQTASIHNATIDSSIYGRPDSISTDMLPYLSDELKKPPTA
15	1051	LLSADRLFMEQEVHPLRSNSVLVHPGAGAATNSSMLPEPDFELINSPARN
	1101	VSNNSDNVAISGNASTISFNQLDMNFDDQATIGQKIQEQPASKSANTVRG
	1151	DDDGLASAPETPRTPTKKESISSKPAKLSSASPRKSPIKIGSPVRVIKKN
	1201	GSIAGIEPIPKATHKPKKSFQGNEISNHKVRDGGISPSSGSEHQHNPSM
	1251	VSVPSQYTDATSTVPDENKDVQHKPREKQKQKHHHRHHHHHKQKTDIPG
20	1301	VVDDEIPDVGLQERGKLFVRVLGIKNINLPDINTHKGRFTLTLDNGVHCV
	1351	TTPEYNMDDHNVAIGKEFELTVADSLEFILTLKASYEKPRGTLVEVTEKK
	1401	VVKSRNRLSRLFGSKDIIITTTKFVPTEVKDTWANKFAPDGSFARCYIDLQ
	1451	QFEDQITGKASQFDLNCFNWETMSNGNQPMKRGKPYKIAQLEVKMLYVP
	1501	RSDPREILPTSIRSAYESINELNNEQNNYFEGYLBQEGGDCPIFKKRFFK
25	1551	LMGTSLLAHSEISHKTRAKINLSKVVDLIYVDKENIDRSNHRNFSVLLL
	1601	DHAFKIKFANGELIDFCAPNKHEMKIWIQNLQEIYRNRFRRQPWVNLML
	1651	QQQQQQQQQQSSQQ

Functional Domains

- 30 A 236 amino acid sequence near the amino terminus of the gene product (α Int1p) is shown in Table 3 (SEQ ID NO:3). This sequence, or a portion thereof, is believed to encompass the ligand binding site, or a portion thereof, and would provide very useful antibodies or could be used as a vaccine antigen itself.

TABLE 3

		SDEDTNASVPPTPLHTTKPTFAQLLNKNNEVN
251	SEPEAL	TDMKLNKRENF
301	QDASKNKTNENI	HNLSFALKAPKNDIENPLNSLTNADISLRSSGSSQSSL
5	351	QSLRNDNRVLESVPGSPKKVNPGLSLNDGIKGFSEVVESLLPRDLSRDK
401	LETTKEHDAPEHNNENF	IDAKSTNTNKGQLLVSSDDHLDSFDRSYNHTEQ
451	SIL	

The following peptide sequences were used as antigens for the preparation of anti-peptide polyclonal antibodies in rabbits by commercial contract through Cocalico Biologicals (Reamstown, PA). The sequences B-F are listed below and correspond to the protein sequence of α Int1p as reported in GenBank, with the exception of one amino acid substitution in sequence (B), as noted below.

B. A 23-mer peptide encompassing the first cation-binding site. This peptide was synthesized by BioSynthesis Inc. (Lewisville, TX). Note that the peptide sequence is MDL, while the GenBank sequence is MDS.

YLS PTN NNN SKN VSD MDL HLQ NL (SEQ ID NO:4)

C. A 23-mer peptide encompassing the second divalent cation-binding site. This peptide was synthesized by BioSynthesis Inc. (Lewisville, TX).

DWK LED SND GDR EDN DDI SRF EK (SEQ ID NO:5)

D. A 17-mer peptide spanning the RGD site and flanking residues. This peptide was synthesized by the Microchemical Facility of the University of Minnesota.

SKS ANT VRG DDD GLA SA (SEQ ID NO:6)

E. A 17-mer peptide from the MIDAS motif of α Int1p. This peptide was synthesized by the Microchemical Facility of the University of Minnesota.

DHL DSF DRS YNH TEQ SI (SEQ ID NO:7)

F. A 17-mer peptide from the C-terminus of α Int1p. This peptide was synthesized by the Microchemical Facility of the University of Minnesota.

WIQ NLQ EII YRN RFR RQ (SEQ ID NO:8)

30

Preparation and Evaluation of Antibodies

Polyclonal antibodies were prepared by Cocalico Biologics (Reamstown,

PA) using the peptides B-F (SEQ ID NOS:4-8) listed above. Generally, each peptide is coupled to an adjuvant, the peptide-adjuvant mixture is injected into rabbits, and the rabbit receives booster injections of the same mixture every three-four weeks. Rabbit serum is withdrawn three weeks after the injections and tested for its titer against the original peptide.

One rabbit each was used to raise antibodies to each individual peptide. IgG antibodies were purified from the respective rabbit's antiserum by affinity purification on a Protein A-Sepharose column (BioRad) according to standard methods. In Figures 1 and 2, anti-Ca denotes antibodies raised to the 23-mer peptide (SEQ ID NO:5) encompassing the second divalent cation binding site; anti-RGD denotes antibodies to the 17-mer peptide encompassing the RGD site and flanking residues (SEQ ID NO:6). CAI-4 denotes the strain of *C. albicans* that was employed. Anti-Ca or anti-RGD antibodies in a concentration of 1.0 mg/ml were incubated with 1×10^6 [35 S]-methionine-labeled *C. albicans* blastospores for 30 minutes on ice at 4°C. Antibody-coated *C. albicans* blastospores were then incubated with confluent monolayers of HeLa cells in a 24-well microtiter plate for 60 minutes at 37°C in 5% CO₂, as described in a previous publication (Bendel and Hostetter, *Journal of Clinical Investigation*, 92, 1840-1849 (1993)). Removal of non-adherent *C. albicans* blastospores, release of the HeLa monolayer with attached *C. albicans* blastospores, counting of the radiolabel, calculation of specific adhesion, and controls for non-specific adhesion were all performed according to the methods in the publication cited above. For Figure 2, methods remained the same, save that CHO cell monolayers (Chinese hamster ovary cells, a second epithelial cell line) were substituted for HeLa cell monolayers.

Figure 1 shows that the antibodies against the second divalent cation binding site (SEQ ID NO:5) or the RGD site and flanking residues (SEQ ID NO:6) inhibit binding to HeLa cells by about 50%. Figure 2 shows that antibodies against the second divalent cation binding site or the RGD site inhibit binding to CHO cells by about 50%.

Induction of α Int1p-Dependent Germ Tubes in Saccharomyces cerevisiae

The entire open reading frame of *α NT1* (*Bgl*III/*Sal*I fragment) was

subcloned into the plasmid pBM272 (obtained from Dr. James Bodley, University of Minnesota) after digestion with *Bam*HI and *Sall*, in order to place the *GALI-10* promoter upstream of the α *INT1* start codon. This plasmid was named pCG01. *S. cerevisiae* YPH500, obtained from the Yeast Genetic Stock Center (Berkeley, CA), was transformed with pBM272 or pCG01 by the lithium acetate procedure as disclosed in Ito et al., *J. Bacteriol.*, 153(1):163-168 (1983). Transformants were selected on agar-based minimal medium (MM = 0.17% yeast nitrogen base/0.5% ammonium sulfate) with 2% glucose, in the absence of uracil. Induction of *aINT1* was achieved by growing transformants containing pCG01 to mid-exponential phase in non-inducing, non-repressing medium (MM without uracil with 2% raffinose) at 30°C, then harvesting, washing, and resuspending them in inducing medium (MM without uracil with 2% galactose) at 30°C for the expression of *aINT1*. YPH500 and YPH500 transformed with vector alone (pBM272) were grown under the identical conditions. *S. cerevisiae* transformants expressing α Int1p from the plasmid pCG01 made abundant germ tubes after 6 hours' growth in inducing medium.

Applicants hereby incorporate-by-reference into the specification the accompanying Sequence Listing as required by 37 C.F.R. §1.52(e)(5).

It will be appreciated by those skilled in the art that various modifications can be made to the above described embodiments of the invention without departing from the essential nature thereof. The invention is intended to encompass all such modifications within the scope of the appended claims. All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference.